Appl. Serial No. 10/763,380 Amdt. Dated August 31, 2009 Response to office action dated March 2, 2009

## Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

## Listing of Claims:

Claims 1-41 (cancelled).

42 (currently amended): A method for the expression of a heterologous polypeptide by a host cell said method comprising:

- a) introducing into a host cell a chimeric nucleic acid sequence comprising:
  - a first nucleic acid sequence capable of regulating the transcription in said host cell of
  - a second nucleic acid sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) <u>a)</u> a nucleic acid sequence encoding at least the N terminal domain and hydrophobic domain of an oil body protein an oleosin protein having the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 or b) a nucleic acid encoding a caleosin protein and having the nucleic acid sequence shown in SEQ ID NO:35 to provide targeting of the fusion polypeptide to a lipid phase linked in reading frame to (ii) a nucleic acid sequence encoding the heterologous polypeptide; and
  - a third nucleic acid sequence encoding a termination region functional in the host cell; and
- b) growing said host cell to produce the fusion polypeptide.

43 (previously presented): The method according to claim 42 further including separating the recombinant fusion polypeptide from cellular host cell components by selective partitioning into a lipid phase.

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44 (previously presented): The method according to claim 43 wherein said selective partitioning comprises centrifugation, floatation or size exclusion.

45 (previously presented): The method according to claim 42 further including separating the recombinant fusion polypeptide from cellular host components by selective partitioning into a lipid phase comprising oil bodies.

46 (previously presented): The method according to claim 45 wherein said recombinant fusion polypeptide is separated by addition of oil body components and reconstitution of the oil bodies.

47 (previously presented): The method according to claim 43 further comprising releasing the heterologous polypeptide from the fusion polypeptide associated with the lipid phase, said method comprising:

- c) including in said second nucleic acid sequence (2) between said nucleic acid sequence (i) encoding the oil body protein and the nucleic acid sequence (ii) encoding the heterologous polypeptide, a linker nucleic acid sequence (iii) encoding an amino acid sequence that is specifically cleavable by enzymatic or chemical means; and
- d) contacting the lipid phase with said enzymatic or chemical means such that said heterologous polypeptide is released from the fusion polypeptide.

48 (previously presented): The method according to claim 47 wherein said linker nucleic acid sequence encodes an amino acid sequence that is recognizable by the proteolytic action of an enzyme selected from the group consisting of thrombin, factor Xa, collagenase, chymosin, clostrapain and viral protease.

49 (previously presented): The method according to claim 47 wherein said enzymatic means comprises an enzyme that is immobilized.

50 (previously presented): The method according to claim 49 wherein said enzyme is immobilized by attachment to an oil body protein that is associated with an oil body.

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51 (withdrawn): The method according to claim 42 wherein said recombinant polypeptide is an enzyme.

52 (withdrawn): The method according to claim 51 wherein said recombinant polypeptide is an enzyme that retains its enzymatic properties while part of the fusion polypeptide is associated with the oil body.

53 (currently amended): A method of preparing an enzyme in a host cell in association with an oil body and releasing said enzyme from the oil body, said method comprising:

- a) transforming a host cell with a chimeric nucleic acid sequence comprising:
  - 1) a first nucleic acid sequence capable of regulating the transcription of
  - a second nucleic acid sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) a) a nucleic acid sequence encoding at least the N-terminal domain and hydrophebic domain of an oil-bedy protein an oleosin protein having the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 or b) a nucleic acid encoding a caleosin protein and having the nucleic acid sequence shown in SEQ ID NO:34 or SEQ ID NO:35 to provide targeting of the fusion polypeptide to an oil body; (ii) a nucleic acid sequence encoding an enzyme and (iii) a linker nucleic acid sequence located between said nucleic acid sequence (i) encoding the oil body and said nucleic acid sequence (ii) encoding the enzyme and encoding an amino acid sequence that is cleavable by the enzyme encoded by the nucleic acid sequence (ii); and
  - a third nucleic acid sequence encoding a termination region functional in said host cell
- b) growing the host cell to produce the fusion polypeptide under conditions such that enzyme is not active:
  - c) recovering the oil bodies containing the fusion polypeptide; and

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d) altering the environment of the oil bodies such that the enzyme is activated and

cleaves itself from the fusion polypeptide.

54 (withdrawn): The method according to claim 53 wherein said enzyme is activated by

lowering the pH or altering the temperature of the oil body environment.

55 (withdrawn): The method according to claim 42 wherein said heterologous

polypeptide is selected from the group consisting of antibodies, glycanases, hormones,

proteases, protease inhibitors and seed storage proteins.

56 (previously presented): The method according to claim 42 wherein said

heterologous polypeptide is selected from the group consisting of a thrombin inhibitor,

hirudin, an interleuken, chymosin, cystatin, xylanase, carp growth hormone, zein, an

antibody and a collagenase.

57 (previously presented): The method according to claim 42 wherein said host cell is

an insect or animal cell.

58 (cancelled).

59 (currently amended): A method according to claim 58-42 wherein said oil body

protein is-nucleic acid sequence (i) encodes an oleosin or a caleosinand has the nucleic

acid sequence shown in SEQ ID NO:1 or SEQ ID NO:4.

60 (currently amended): A method for the expression of a heterologous polypeptide

by a host cell said method comprising:

a) generating by homologous recombination into a host cell a chimeric nucleic acid

sequence comprising:

1) a first nucleic acid sequence capable of regulating transcription in said host

cell

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- 2) a second nucleic acid sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) <u>a)</u> a nucleic acid sequence encoding at least the N-terminal domain-and hydrophobic domain-of-an-oil-body-protein an oleosin protein having the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 or b) a nucleic acid encoding a caleosin protein and having the nucleic acid sequence shown in SEQ ID NO:35 to provide targeting of the fusion polypeptide to a lipid phase, linked in reading frame to (ii) a nucleic acid sequence encoding the heterologous polypeptide; and
- a third nucleic acid sequence encoding a termination region functional in the host cell; and
- b) growing said host cell to produce the heterologous polypeptide.

61 (currently amended): A chimeric nucleic acid sequence, capable of being expressed in association with an oil body of a host cell comprising:

- a first nucleic acid sequence capable of regulating the transcription in said host cell of
- a second nucleic acid sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) a) a nucleic acid sequence encoding at least the N-terminal-domain and hydrophobic domain of an oil-bedy-protein an oleosin protein having the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 or b) a nucleic acid encoding a caleosin protein and having the nucleic acid sequence shown in SEQ ID NO:35 to provide targeting of the fusion polypeptide to a lipid phase linked in reading frame to (ii) a nucleic acid sequence encoding the heterologous polypeptide; and
- a third nucleic acid sequence encoding a termination region functional in the host cell

62 (withdrawn): The chimeric nucleic acid sequence according to claim 61 wherein said nucleic acid sequence (ii) encodes an enzyme.

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63 (previously presented): The chimeric nucleic acid sequence according to claim 61 further including (iii) a linker nucleic acid sequence encoding an amino acid sequence

that is specifically cleavable by enzymatic means wherein said linker nucleic acid sequence (iii) is located between said (i) nucleic acid sequence encoding the oil body

protein and said (ii) nucleic acid sequence encoding the heterologous polypeptide.

64 (previously presented): The chimeric nucleic acid according to claim 63 wherein said linker nucleic acid sequence (iii) encodes a cleavage site for an enzyme selected from

the group consisting of thrombin, factor Xa, collagenase chymosin and viral protease.

65 (cancelled).

66 (currently amended): A chimeric nucleic acid sequence according to claim 61 wherein said eil-bedy-protein-gene-is-nucleic acid sequence (i) encodes an oleosin er-a

ealeosinand has the nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:4.

67 (previously presented): An expression cassette comprising a chimeric nucleic acid

sequence according to claim 61.

68-69 (cancelled).

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